Environmental Health Perspectives
Vol. 57, pp. 33-41, 1984

Ethylene Glycol Monoethyl Ether and Ethylene Glycol Monoethyl Ether Acetate Teratology Studies

by J. E. Doe*

An inhalation teratology study was conducted in rats at 10, 50 and 250 ppm ethylene glycol monoethyl ether (EGEE) and in rabbits at 10, 50 and 175 ppm EGEE. This study was designed to supplement a study conducted for NIOSH which showed teratogenic effects in rats at 200 ppm EGEE and in rabbits at about 160 ppm EE. In this study, EGEE was found not to cause teratogenic effects at concentrations up to and including 250 ppm in rats and 50 ppm in rabbits, while 175 ppm EGEE was considered to be a marginal effect level for teratogenic effects in rabbits. Fetotoxicity was observed at 250 ppm EGEE and possibly at 50 ppm EGEE in rats.

An inhalation teratology study using ethylene glycol monoethyl ether acetate (EGEEA) has been conducted in rabbits at 25, 100 and 400 ppm. There was evidence of teratogenicity (vertebral malformations) at 400 ppm EGEEA and slight fetotoxicity at 100 ppm; 25 ppm was a no effect level.

Introduction

Ethylene glycol monoethyl ether, 2-ethoxyethanol (EGEE) is a solvent for celluloses, acrylics, dyes, inks, resins and varnishes. EGEE has a boiling point of 135°C at 760 mm/Hg and a vapor pressure of 4 mm/Hg at 20°C. Therefore, inhalation could be a major route of exposure for users of this material. In a previously reported study (*1*), rats and rabbits were exposed to atmospheres containing EGEE during gestation. Pregnant Wistar rats were exposed to 767 or 202 ppm of EGEE for 7 hr/day on days 1 to 19 of gestation. At 767 ppm, complete embryomortality and maternal toxicity were seen, while at 202 ppm, fetal growth depression and an increased incidence of terata compared with controls were seen. Similar results were found when rabbits were exposed to EGEE at either 617 or 160 ppm of EGEE during gestation. Maternal toxicity and an increase in the incidence of embryomortality were seen at both exposure levels, while at 160 ppm there was an increase in the incidence of terata. Therefore, a no-effect level for either embryotoxicity or teratogenicity was not established in either species. The purpose of the studies reported here was to confirm the teratogenicity seen in the previous study (*1*) and to determine a no-effect level for embryotoxicity and teratogenicity for EGEE.

Ethylene glycol monoethyl ether acetate, 2-ethoxyethanol acetate, (EGEEA) is a solvent for nitrocellulose, low viscosity cellulose and resins. EGEEA has a boiling point of 156°C at 760 mm/Hg and a vapor pressure of 2 mm/Hg at 20°C.

Inhalation could therefore be a route of exposure for users of this material. EGEEA has been reported (*2*) to cause testicular atrophy and leukopenia in mice gavaged for 5 days/week for 5 weeks. This was similar to the effects seen with ethylene glycol monomethyl ether (EGME) and EGEE in the same study. Both EGME (*3*) and EGEE (*1*) have been shown to be teratogenic as well as cause effects on the testis. Therefore, this study was carried out to determine whether EGEEA would also show teratogenicity as well as the effects on the testis. EGEEA has been confirmed to be teratogenic in rats (*4*), and for this reason only a rabbit inhalation teratology study was carried out.

Both the studies on EGEE and the study on EGEEA were sponsored by the Glycol Ethers Program Panel of the Chemical Manufacturers Association, Washington, DC, and reported to them by Tinston et al. (*5–7*).

Materials and Methods

Test Materials

Ethylene glycol monoethyl ether (EGEE) and ethylene glycol monoethyl ether acetate (EGEEA) were both supplied by Imperial Chemical Industries PLC, Petrochemicals and Plastics Division, Wilton, Middlesbrough, UK. Both materials were more than 99% pure.

*Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Animals

Rats. Nulliparous specific pathogen-free female rats of the Alpk/AP (Wistar-derived) strain were used with a weight range of 200 to 280 g. The animals were approximately 11 to 13 weeks old. The rats were paired overnight and the following morning the detection of spermatozoa in vaginal smears was used as evidence of mating. The day on which spermatozoa were detected was termed day 0 of pregnancy.

Rabbits. Virgin female Dutch rabbits (5–7 months old, 1.7–2.8 kg in weight) were supplied by Ranch Rabbits, Crawley Down, Sussex, UK, and housed individually in cages. They were acclimatized to the exposure chambers, where they were individually housed in wire mesh cages for no more than 6 hr/day for at least 6 days prior to mating, during which time the clinical condition of each rabbit was monitored. Twelve bucks of the same strain and of proven fertility supplied by the same breeder were housed within the same experimental unit. Each doe was placed with the buck and coitus observed, a vaginal smear was taken from the first doe each buck mated. The smear was examined for the presence of motile sperm. Within 5 hr of mating, each doe received an intravenous injection of 25 IU of chorionic gonadotrophin (Pregnyl, Organon Laboratories Limited, Morden, Surrey, UK) to promote ovulation. Day of mating was termed day 0 of pregnancy. Following mating the does were placed in exposure chambers for 6 hr/day, but were not exposed to test material until day 6 of gestation.

Exposure Chambers

The exposure chambers (*8*) had an internal volume of approximately 3.4 m^3. They were constructed of stainless steel and access was gained to each chamber through a door fitted with a safety glass window. Air entered at the front of each chamber and was extracted at the back. Within each chamber were 6 cage levels and excreta collection trays which rotated concurrently (0.5 times/min) with the direction of flow of the input air. In each chamber, each of the levels supported four cages and an air flow rate in each in each chamber of 600 L/min was used. The chamber air supply was conditioned to 22°C and 50% relative humidity, and the temperature and relative humidity in each chamber were recorded daily. The distribution of both EGEE and EGEEA at equilibrium within the chambers was found to be within ± 5% of the nominal concentration.

Atmosphere Generation and Analysis

Atmospheres of both EGEE and EGEEA were generated by metering appropriate amounts into a condenser at 34 to 40°C and passing the vaporized test compound into the input air of each chamber. The concentrations of test substance in each chamber were analyzed 9 to 14 times per exposure period through 4 mm internal diameter copper tubing leading to a central analyzer. The atmospheric concentrations were monitored by using Wilks-Miran infrared analyzers with the following conditions: EGEE, wavelength 8.9 μm, slit width 1 mm; EGEEA, wavelength 9.2 μm, slit width 1 mm. The pathlengths were adjusted according to the concentration within a chamber.

Maternal Observations

Rats. The body weight of each rat was recorded on day 0, 5, 6 to 15, 16 and 21. Food consumption was also assessed over the same time period. Each animal was observed daily for changes in clinical condition and during the exposure period for any abnormalities.

Rabbits. The body weight of each rabbit was recorded on day 0, daily on days 5 to 19 and on days 24 and 28. Food consumption was also measured over this time period. The clinical condition of all animals was recorded daily and they were observed during each exposure for any abnormalities.

Terminal Investigations

Rats. On day 21 of pregnancy the rats were killed by an overdose of Halothane BP and subjected to a postmortem examination. Blood samples were taken by cardiac puncture and placed in EDTA pots for hematological assessment. Thymus and spleen were weighed. The uterus of each rat was dissected out and the gravid uterus weighed. The number of corpora lutea in each ovary was counted. The uterus was then opened by an incision on the abendometrial wall and the number of implantations of early and late uterine deaths were counted. Intrauterine deaths were identified as being late when fetal tissues were distinguishable. Each live fetus was removed from the uterus by severing the umbilical cord. It was then weighed and examined externally for gross abnormalities, including cleft palate. Approximately half of the fetuses in each litter were fixed in 70% methanol. These fetuses were subsequently eviscerated and stained with Alizarin Red S for skeletal examination. The remaining fetuses in each litter were fixed in Bouin's fluid to be processed for visceral examination.

Rabbits. Rabbits were killed on day 29 of pregnancy with an intravenous injection of pentobarbitone sodium BP and subjected to a postmortem examination. Blood samples were taken by cardiac puncture for subsequent hematological analysis and femoral bone marrow smears were prepared. The spleens were weighed. The uterus was dissected out, the gravid uterus was weighed, and the number of corpora lutea in each ovary was counted. The uterus was opened by an incision on the abendometrial wall, and the number of implantations, early intrauterine deaths and late intrauterine deaths were counted. Intrauterine deaths were identified as being late when fetal tissues were distinguishable. Each live fetus was removed from the uterus by severing the umbilical cord, and each fetus was then weighed and examined externally for gross defects, including cleft palate.

Assessment of Fetuses

Rats. Visceral assessment of the methanol-fixed fetuses consisted of examination of the abdominal and thoracic contents. At this time, the sex of each fetus was determined externally and confirmed by internal examination. Most fetuses fixed in Bouin's fluid were stored until decalcification was complete. They were then transferred to 70% methanol, and the head and thorax of each fetus was sectioned at approximately 1.5 to 2 mm intervals and the sections examined under a stereo microscope (*9*). The abdominal organs were examined *in situ*. Skeletons of all fetuses fixed in methanol and stained with Alizarin Red S were examined under a stereo microscope to assess morphological development and the degree of ossification. The individual bones of the manus and pes were assessed and the results converted into a four-point scale. Defects were classified according to the scheme: major (rare or possibly lethal or both) or minor deviations from normal (deviations from normal that are common at external, visceral or skeletal examination) defects. Variations in the degree of ossification in the fetuses were also recorded and classified as minor defects or variants, depending upon frequency of occurrence in historical control. Extrathoracic ribs were classified as variants.

Rabbits. During visceral examination, the skin was removed from each fetus and the eyes examined for opacities, the major organs of the abdomen and thorax were examined *in situ* for defects, and the sex of the fetus was determined. The position of major blood vessels in the thorax and their emergence from the heart were noted. A section across the heart was made to check for any ventricular septal defects. All fetuses were eviscerated and placed in 70% methanol. After approximately 24 hr an incision was made along the fronto-parietal suture line of each fetus in order to examine the brain. All fetuses were then examined with Alizarin Red S (*10*) and examined to assess morphological development and the degree of ossification. The individual bones of the manus and pes were assessed and the result converted to a five-point scale.

Study Designs

The exposure concentrations and number of rats used in the rat EGEE study are shown in Table 1. The rats were exposed to the appropriate concentration of EGEE for 6 hr/day on days 6 to 15 inclusive of gestation.

Exposure concentrations and number of rabbits used in the rabbit EGEE study are shown in Table 2. The rabbits were exposed on days 6 to 18 inclusive of gestation to the appropriate concentration of EGEE for 6 hr/day.

Exposure concentrations and number of rabbits used in the rabbit EGEEA study are shown in Table 3. The rabbits were exposed to the appropriate concentration of EGEEA on days 6 to 18 of gestation inclusive for 6 hr/day.

Results

Rat EGEE Study

Atmosphere Concentrations. The overall atmosphere concentrations were: 9.9 ± 0.9 ppm; 50.8 ± 2.3 ppm; 249.2 ± 10.4 ppm.

Maternal Observations. There were no treatment-related effects upon either body weight or food consumption. There were also no clinical changes in clinical condition of the rats caused by exposure to EGEE. Postmortem examination showed that the spleen and thymus weights were not affected by exposure to EGEE, and there were no other treatment-related abnormalities. In the rats exposed to 250 ppm EGEE there were reductions in hemoglobin, hematocrit and mean cell volume in the red blood cells, but there were no effects at either 50 or 10 ppm of EGEE.

Litter Data. There was a higher level of pre-implantation loss in all exposed groups compared with controls, although this was statistically significant only in the 10 and 50 ppm groups (Table 4). Post-implantation losses were also slightly higher in the exposed groups compared with controls, but these differences were not

Table 1. Rat EGEE study.

Group no.	Exposure concentration of EGEE, ppm	Number of rats
1	Control	24
2	10	24
3	50	24
4	250	24

Table 2. Rabbit EGEE study.

Group no.	Exposure concentration of EGEE, ppm	Number of rabbits
1	Control	24
2	10	24
3	50	24
4	175	24

Table 3. Rabbit EGEEA study.

Group no.	Exposure concentration of EGEEA, ppm	Number of rabbits
1	Control	24
2	25	24
3	100	24
4	400	24

Table 4. Rat EGEE study.

	Exposure concentration of EGEE			
	Control	10 ppm	50 ppm	250 ppm
No. pregnant	23/24	24/24	23/24	21/24
Pre-implantation loss, %	2.4	9.7	14.3*	6.2
Post-implantation loss, %	5.5	7.6	8.9	12.6
Mean no. of live fetuses	12.2	10.6*	10.8*	11.1
Mean live fetus weight, g	5.1	5.2	5.1	4.7

*Statistically significantly different from the controls, $p < 0.05$.

Table 5. Rat EGEE study: comparison of fetal visceral, external and skeletal defects.

	Exposure concentration of EGEE			
	Control	10 ppm	50 ppm	250 ppm
External and visceral defects				
No. (%) showing any minor defects	33 (11.7)	41 (16.1)	29 (11.6)	43 (18.4)*
No. (%) showing any major defects	0	0	0	1 (0.4)
Skeletal defects:				
No. (%) showing any minor defects	68 (46.3)	52 (39.7)	66 (51.2)	119 (97.5)*
No. (%) showing any major defects	0	0	0	0

*Statistically significantly different from the controls $p < 0.05$.

Table 6. Rat EGEE study: incidence of specific visceral and external defects.

	Exposure concentration of EGEE			
	Control	10 ppm	50 ppm	250 ppm
Minor defects				
No. (%) renal pelvic dilation	19 (6.8)	25 (9.8)	22 (9.8)	30 (12.8)*
No. (%) hydroureter	13 (4.6)	26 (2.4)	4 (1.6)	10 (4.3)
No. (%) bladder distended	0	1 (0.4)	0	0
No. (%) dermal hemorrhage	2 (0.7)	2 (0.8)	0	0
No. (%) limb malrotation	0	9 (3.5)*	2 (0.8)	3 (1.3)
No. (%) lateral ventricles of brain dilated	0	0	1 (0.8)	0
Major defects				
Right uterer/kidney fused to left kidney, right kidney vestigial	0	0	0	1 (0.4)

*Statistically significantly different from the controls, $p < 0.05$.

statistically significant and were within historical control values of 2 to 17% in this strain of rat. The proportion of dams with any late intrauterine death and the mean percentage late intrauterine deaths was statistically significantly increased in the 250 ppm group. The mean number of live fetuses was reduced in the exposed groups compared with the controls, and this was statistically significant in the 10 and 50 ppm groups but not at 250 ppm. The lower litter sizes of the exposed groups were reflected in reduced mean gravid uterus weights and in reduced total litter weights. Total litter weight was statistically significantly reduced in the 10 and 250 ppm groups, the reduction being most marked at 250 ppm. While there was no effect on mean fetal weight in the 10 and 50 ppm group, this parameter was statistically significantly reduced in the 250 ppm group.

Fetal, Visceral, External and Skeletal Defects. The incidence of minor external and visceral defects was slightly elevated in the 10 and 250 ppm groups (Tables 5 and 6). The proportion of fetuses affected was statistically significantly higher in the 250 ppm group than in the controls, but when considered on a litter basis the increase was not statistically significant. The increases were due to a higher incidence of limb malrotation in the 10 ppm group and pelvic dilatation of the kidney in the 250 ppm group. No cardiovascular abnormalities were seen. There was an increased incidence of minor skeletal defects in the 250 ppm group, a proportion of fetuses having these defects being statistically significantly increased. Examination of the specific skeletal findings showed this to be the consequence of increased partial/nonossification of parts of the skull, the thoracic centra, the lumbar centra, the lumbar vertebrae and sternebrae, increased sternebrae abnormalities and increased incidence of 27 presacral vertebrae. There was little evidence of any increased incidence of these effects in the 10 and 50 ppm groups, the only statistically significant increases being the proportion of fetuses with partially ossified lumbar vertebral process in the 10 ppm group and partially ossified second sternebrae and unossified cervical centra in the

Table 7. Rabbit EGEE study: litter data.

	Exposure concentration of EGEE			
	Control	10 ppm	50 ppm	175 ppm
No. pregnant and included in analyses	21/24	21/24	16/24	22/24
Pre-implantation loss, %	19.5	17.6	22.1	25.7
Post-implantation loss, %	5.7	8.4	8.8	5.7
Mean no. of live fetuses	6.5	6.6	6.0	6.1
Mean live fetus weight, g	34.1	34.2	35.7	36.1

Table 8. Rabbit EGEE study: comparison of fetal visceral, external and skeletal defects.

	Exposure concentration of EGEE			
	Control	10 ppm	50 ppm	175 ppm
External and visceral defects				
No. (%) showing any minor defects	6 (4.4)	8 (5.8)	4 (4.2)	2 (1.5)
No. (%) showing any major defect	1 (0.7)	1 (0.7)	0	2 (1.5)
Skeletal defects				
No. (%) showing any minor defects	44 (32.4)	72 (52.2)	35 (36.5)	87 (64.5)*
No. (%) skeletal variants	70 (51.5)	84 (60.0)	62 (64.6)	106 (79.1)*

*Statistically significantly different from the controls, $p < 0.05$.

Table 9. Rabbit EGEE study: incidence of major visceral effects.

	Exposure concentration of EGEE			
	Control	10 ppm	50 ppm	175 ppm
Right subclavian artery, absent, aorta and heart reduced in size (%)	0	0	0	1 (0.7)
Extreme pelvic dilatation of both kidneys	1 (0.7)	0	0	0
Umbilical hernia	0	0	0	1 (0.7)

50 ppm group. The development of manus and pes were affected in the 250 ppm group, the mean scores per fetus being significantly increased. There was no effect on manus and pes development in the 10 and 50 ppm groups.

Rabbit EGEE Study

Atmosphere Analysis. The daily mean analyzed concentration of EGEE were all within 10% of the target concentration except for 2 days and the overall concentrations were 10.1 ± 0.03 ppm, 51 ± 4 ppm and 175 ± 3 ppm.

Maternal Observations. There were no effects due to compound on body weight gain or food consumption and no clinical abnormalities which could be attributed to exposure to EGEE.

Litter Data. There was no evidence for any treatment-related effects on the litter data (Table 7).

Fetal, External, Visceral and Skeletal Defects. There were two major defects in the 175 ppm group: one fetus had a cardiovascular defect (heart and aorta reduced in size and the right subclavian artery was missing), and another fetus had a ventral wall defect (umbilical hernia) (Tables 8 and 9). There was one fetus in the 10 ppm group with a major defect (right gonad appeared male, and left gonad appeared female; this defect was not found in the other two groups). One fetus in the control group had a major defect (extreme pelvic dilatation of the kidney). The incidence of skeletal defects was statistically significantly greater in the 175 ppm than in the control group, largely as a result of retarded ossification of the skeleton and an increased incidence of 27 presacral vertebrae. The incidence of minor skeletal defects was also increased in the 10 ppm group in comparison with the control group but is not considered to be treatment-related, as it was not statistically significant and there was only a very slight increase observed in the 50 ppm group. The incidence of skeletal variants in the 175 ppm group was statistically significantly greater than in the control group and this was entirely due to an increased number of fetuses with extra ribs, both short and of normal length. The incidence of skeletal variants was slightly increased in the 10 and 50 ppm groups compared with controls, due to an increase in the incidence of extra ribs and partially ossified 5th vertebrae. Since the incidence of extra ribs was not statistically significantly increased at either 10 or 50 ppm and the slightly increased incidence of partially ossified 5th sternebrae was not dose-related, these two specific defects were considered not to be related to exposure to EGEE at 10 ppm. There was no effect due to exposure on the mean manus and pes development scores.

EGEEA Rabbit Study

Atmosphere Analysis. The overall mean atmosphere concentrations were 24.9 ± 0.7 ppm, 99 ± 2 ppm, 412 ± 7 ppm.

Maternal Observations. There was a dose-related adverse effect on body weight in all three groups, which was only statistically significant in the rabbits exposed to 400 ppm during the first few days of exposure. There was a dose-related decrease in food consumption, but this was also only statistically significant in the 400 ppm group. There were no clinical abnormalities which could be related to exposure to EGEEA in any of the three groups. There was no effect on spleen weight at postmortem, nor were there any other maternal macroscopic observations which could be related to EGEEA exposure. There was a statistically significant reduction in hemoglobin concentration and a slight reduction in the associated red blood cell parameters in the rabbits exposed to 400 ppm.

Litter Data. There was an increased incidence of intrauterine deaths in the 400 ppm group which was associated with a higher percentage post-implantation loss and the lower number of live fetuses per litter (Table 10). When the data from three animals with total resorptions were omitted from the 400 ppm group, there were no statistically significant differences in percentage post-implantation loss. Mean live fetal weight was statistically significantly reduced in the 100 and 400 ppm groups. There were no statistically significant differences from control values in the 25 ppm group.

Fetal, External, Visceral and Skeletal Defects. The incidence of fetuses with major defects (Tables 11 and 12) was: control group 1; 25 ppm group, 1; 100 ppm group, 2; and 400 ppm group, 8.

In the 400 ppm group the fetuses with major defects were from four different litters: two of the eight fetuses had major external or visceral defects (brain ventricles moderately dilated and both forelimbs malrotated) and five had major skeletal defects associated with the vertebral column with misaligned vertical arches and additional hemivertebrae. One fetus had a major visceral defect (agenesis of the right kidney) and a major skeletal defect of a similar nature to the others. There were no major skeletal defects in the 25 or 100 ppm EGEEA groups, but one fetus from the 25 ppm group

Table 10. Rabbit EGEEA study: litter data.

	Exposure concentration of EGEE			
	Control	25 ppm	100 ppm	400 ppm
No. pregnant	16/24	15/24	17/24	19/24
Pre-implantation loss, %	14.0	19.1	8.2	12.3
Post-implantation loss, %	9.4	18.1	6.7	24.4*
Mean no. of live fetuses	6.6	5.1	7.4	4.9*
Mean total litter weight, g	230	194	223	179*
Mean live fetal weight, g	34.7	35.2	30.1*	30.8*

*Statistically significantly different from the controls, $p < 0.05$.

Table 11. Rabbit EGEEA study: comparison of fetal visceral, external and skeletal defects.

	Exposure concentration of EGEE			
	Control	25 ppm	100 ppm	400 ppm
External and visceral				
No. (%) showing minor defects	22 (20.8)	18 (23.4)	30 (27.0)	51 (54.8)*
No. (%) showing major defects	0 (0)	1 (1.3)	2 (1.8)	3 (3.2)
Skeletal				
No. (%) variants	50 (47.2)	40 (51.9)	72 (64.9)*	92 (98.9)*
No. (%) showing any defects	23 (21.7)	14 (18.2)	37 (33.3)*	91 (97.8)*
No. (%) showing major defects	1 (0.9)	0	0	6 (6.5)*

*Statistically significantly different from the controls, $p < 0.05$.

Table 12. Rabbit EGEEA study: incidence of major defects.

	Exposure concentration of EGEEA			
	Control	25 ppm	100 ppm	400 ppm
No. (%) with moderate dilatation of brain ventricles	0	0	0	1 (1.1)
No. (%) with absent kidney	0	1 (1.3)	0	1 (1.1)
No. (%) with misplaced ovary	0	0	1 (0.9)	0
No. (%) with forelimb malrotated	0	0	1 (0.9)	1 (1.1)
No. (%) with multiple defects of vertebrae	1 (0.9)	0	0	6 (6.6)

had agenesis of the left kidney and one fetus in the 100 ppm group had an ovary attached to the intestine. Another fetus in the 100 ppm group had its right forelimb malrotated. In the control group, one fetus had a major vertebral defect. There were no cardiac abnormalities in this study.

In the 400 ppm group there was a statistically significant increase in the proportion of fetuses with minor external/visceral defects in comparison with the control group. Within the 400 ppm group, there was a statistically significant increase in the proportion of fetuses with pelvic dilation, opaque/empty gall bladders, pale and reduced spleens. In the 100 ppm group the proportion of fetuses with pale spleen was also statistically significantly increased in comparison with the control group. There were no statistically significant increases in the proportion of fetuses with any specific external or visceral defect in the 25 ppm group. The proportion of fetuses with any external or visceral defects was statistically significant only in the 400 ppm group. In addition there was a statistically significant increase in the proportion of fetuses with minor skeletal defects in the 400 ppm group which was indicative of retarded ossification. In the 100 ppm group, the proportion of fetuses with minor skeletal defects were slightly increased but not significantly. Two specific defects: partial ossification of the first cervical centrum and the second sternebrae were statistically significantly higher in the 100 ppm group. The incidence of one minor defect, an extra center of ossification above the first sternebrae, was statistically significantly higher in the 25 ppm group. Statistically significant differences indicating retarded ossification of the manus and pes were observed in the 100 ppm group and for the pes alone in the 400 ppm group. The proportion of fetuses with skeletal variants was statistically significantly higher than the 400 ppm groups. The incidence of 13 bilateral ribs was significantly increased in both groups. There were no statistically significant increases in variants in the 25 ppm group.

Discussion

The only evidence for maternal toxicity in the EGEE rat study was in the 250 ppm group, where statistically significant reductions in hemoglobin, hematocrit and the red cell volume were observed. These changes were slight, but are consistent with the hematological changes seen in other studies with glycol ethers including EGEE (*2,11*). At 250 ppm there was a marked increase in the incidence of late uterine deaths and in the proportion of dams affected, indicating an increased post-implantation loss. There was also a decrease in mean fetal weights in the 250 ppm groups; thus 250 of EGEE caused an increased post-implantation loss and retarded fetal growth, although no effects were seen at 50 or 10 ppm. The slightly increased incidences of fetal visceral and external defects in the 10 and 250 ppm groups were due to limb malrotation in the 10 ppm group and renal pelvic dilatation in the 250 ppm group. There were no statistically significant increases in the incidence of limb malrotation in the 50 and 250 ppm groups, and therefore this defect is considered to have no toxicological significance. The association of renal pelvic dilation with exposure to 250 ppm cannot be precluded, but the defect is a minor one and not indicative of teratogenicity. No major skeletal defects were identified in this study, but overall there was a fetotoxic effect at 250 ppm, indicated by reduced ossification, which could be related to the retarded fetal growth observed at this level. The increased incidence of skeletal variants in the 250 ppm group was also consistent with a fetotoxic effect. A small number of these changes occurred at 50 ppm, i.e., unossified cervical centra and extra ribs, and these could represent a slight fetotoxic effect. Slight fetoxicity is also indicated by partial ossification of the second sternebrae at 50 ppm. The minor changes in ossification of the cervical centra seen at 10 ppm are normally reversible once treatment ceases and are therefore considered to be of minimal toxicological importance. In addition, the increased incidence at 10 ppm was within the historical range for this strain of rat.

The results of the study reported here are similar to those given previously (*1*), where pregnant female rats were exposed to 200 ppm of EGEE for 7 hr/day on days 1 to 19 of gestation. Fetal body weights were reduced, and this effect was accompanied by skeletal defects including retarded ossification. There was also a low incidence of cardiovascular abnormalities: 6 out of 324 fetuses were affected, whereas no cardiac abnormalities were seen in the 234 fetuses exposed to 250 ppm in this study. The exposure levels used in the two studies were very similar, especially when the slightly longer exposure period (7 hr/day compared to 6 hr/day in the previous study) are taken into account. It is unlikely that different lengths of treatment (days 1–19 of gestation versus days 6–15 of gestation) would have influenced the induction of cardiac abnormalities, as the sensitive period of organogenesis is included in both periods. It should be borne in mind that the incidence of cardiac abnormalities in the previous study 6/324, 1.5% is low and 200 to 250 ppm may be close to the threshold for this effect in rats. The data from the present study indicate that EGEE is not teratogenic to rats exposed during organogenesis at concentrations up to and including 250 ppm. It is, however, fetotoxic and shows mild maternal toxicity (hematological changes) at 250 ppm and slight fetotoxicity at 50 ppm.

In the rabbits exposed to EGEE there was no evidence of maternal toxicity in either body weight, food consumption or clinical condition. Although blood cell counts were not carried out, there was no indication of an effect on the bone marrows in the 175 ppm group. There was also no evidence of embryotoxicity or fetotoxicity from the litter data, since fetal weights, numbers of fetuses and the incidence of intrauterine deaths in the groups exposed to EGEE were similar to the controls. There were also no statistically significant increases in the incidence of fetal, external or visceral defects in any of the EGEE exposure levels in the rabbit study, but there was one fetus in the 175 ppm EGEE group with a cardiovascular defect and one other fetus in the same group with an abdominal wall defect.

At 175 ppm EGEE in the rabbit, both the incidence and type of minor skeletal defects and the occurrence of extra rudimentary ribs are considered to be indicative of fetotoxicity. The occurrence of extra normal length ribs is regarded as indicating possible teratogenic potential, which would be expressed at higher levels of the relevant test compound (*13*). There was no incidence of fetotoxicity in the 10 and 50 ppm groups, the slight increase in the incidence of unossified 5th sternebrae at 10 ppm is considered to be coincidental, since similar increases were not apparent at 50 or 175 ppm. Other changes were also not dose-related.

The results reported here are similar again to those reported previously (*1*) when New Zealand White rabbits were exposed to 160 ppm of EGEE for 7 hr/day on days 1 to 19 of gestation. In both the present study and the other study, the incidence of skeletal variants was increased, and there were no effects on fetal weights. The main difference was that in the previous study (*1*) there was an increase in intrauterine mortality, a slight increase in the incidence of a major cardiovascular defect (5 out of 167 fetuses, 3% with fusion of aorta and pulmonary artery) and a slight increase in the incidence of ventral wall defects. There was no evidence for increased intrauterine mortality at 175 ppm in the study reported here; the incidence of fetal major cardiovascular defect (right subclavian artery absent, heart and aorta reduced in size) was 1 out of 134 fetuses (0.7%), and the incidence of ventral wall defect was 1 out of 134 fetuses (0.7%).

While an incidence of major defects of 2 out of 134 fetuses examined would not normally be considered to be evidence of a teratogenic effect, consideration of the other studies where EGEE has been shown to cause cardiac defects (*1*) suggests that 175 ppm could be considered to be a marginal effect level for teratogenicity in this study. However, 50 ppm EGEE was a clear no-effect level for both teratogenicity and fetotoxicity in this study.

Taken overall, the results of the two studies reported here for the effect of EGEE in rats and rabbits indicate that levels of 175 to 250 ppm may be around the threshold level for teratogenicity; 175 to 250 ppm has been shown to be fetotoxic in both rats and rabbits, and 50 ppm is mildly fetotoxic in rats, although there were no effects in rabbits. These studies overall indicate a marginal fetotoxic effect level of 50 ppm and a clear no-effect level of 10 ppm in both species.

Exposure to 400 ppm of EGEEA caused some mild toxicity in the rabbit, since there was an adverse effect on weight gain during the exposure period and there was a marginal reduction in blood hemoglobin concentration in the 400 ppm groups. There was no evidence of maternal toxicity in the pregnant rabbits exposed to either 25 or 100 ppm EGEEA. The occurrence of total litter resorption in three dams in the 400 ppm EGEEA group cannot be conclusively associated with exposure to EGEEA because the post-implantation loss in those females with live fetuses *in utero* was no greater than the controls, indicating that 400 ppm did not decrease the number of fetuses in surviving litters. However, the reduction in mean litter weight as a result of reduced fetal weight is evidence of fetotoxicity at 400 ppm. There was also a reduction in mean fetal weights, but not litter weight, in the group exposed to 100 ppm EGEEA. While the reduction in mean fetal weight may be attributable in part to the higher proportion of litters containing a large number of fetuses with subsequent intralitter competition, it may be evidence of a slight fetotoxic effect at 100 ppm EGEEA. There was no evidence from the litter data of a fetotoxic effect at 25 ppm EGEEA.

Examination of the fetuses from the rabbits exposed to 400 ppm confirmed fetotoxicity as indicated by reduced fetal weight. There was reduced skeletal ossification in both the vertebrae and the sternebrae, increases in the numbers of thoracic ribs and an increased incidence of slight renal pelvic dilatation. Increased incidences of opaque gall bladders and small spleens are difficult to interpret, and the biological significance of these changes are unknown.

There was also some evidence of fetotoxicity from the skeletal examination of the fetuses from the rabbits exposed to 100 ppm EGEEA. There was an increased incidence of extra thoracic ribs and some retarded ossification in the vertebral column and sternebrae which only occurred in statistically significant numbers where there was a greater instance at 400 ppm EGEEA and therefore forming part of a dose-response relationship. None of these effects were seen in the fetuses exposed to 25 ppm and this concentration is therefore not fetotoxic.

At 400 ppm EGEEA there were five fetuses with major skeletal malformations involving the vertebral column; one fetus had a similar major skeletal malformation and agenesis of one kidney; one fetus had moderate dilatation of the brain ventricles, and one had malrotation of the forelimbs. The incidence of six major skeletal malformations compared with one in the controls provides evidence of possible teratogenicity at 400 ppm EGEEA. The incidences of major external and visceral abnormalities do not provide evidence for teratogenicity at 400 ppm EGEEA. There was no evidence of teratogenicity at either 100 ppm or 25 ppm EGEEA.

Although there were no cardiac abnormalities in this study, the results are broadly similar to those reported previously (*4*). Rats were exposed to 600, 390 or 130 ppm of EGEEA. Then there was complete resorption of all implants at 600 ppm EGEEA; at 390 ppm there was fetotoxicity in the form of delayed ossification and cardiac abnormality as evidence of teratogenicity.

However, at 130 ppm EGEEA there was only one major abnormality, a cardiac abnormality, and evidence of fetotoxicity, i.e., an increased incidence of skeletal defects, mainly delayed ossification.

The two studies together therefore indicate that 390 to 400 ppm EGEEA is teratogenic, 100 to 130 ppm EGEEA is fetotoxic and that 25 ppm EGEEA is a no-effect level.

These studies were sponsored by the Chemical Manufacturers Association, Washington, DC.

The studies reported here were the result of collaboration between the following: M. E. Killick, D. J. Tinston, G. A. Wickramaratne, M. H. Litchfield with the aid of M. Kilmartin, D. Kinsey, L. K. Head, S. M. Millward, D. R. Woodcock, I. S. Chart, M. Godley and M. Thomas. Thanks are due to L. Pritchard for typing the manuscript.

REFERENCES

1. Hardin, B. D., Bond, G. P., Sikov, M. R., Andrew, F. D., Beliles, R. P., and Niemeier, R. W. Testing of selected workplace chemicals for teratogenic potential. Scand. J. Work Environ. Health 7 (Suppl. 4): 66–75 (1981).
2. Nagano, K., Nakayama, E., Koyano, M., Dobayaski, H., Adachi, H., and Yamada, T. Testicular atrophy of mice induced by ethylene glycol monoalkyl ethers. Japan. J. Ind. Health 21: 29–33 (1979).
3. Nagano, K., Nakayama, E., Oobayashi, H., Yamada, T., Adachi, H., Nishizawa, T., Ozana, H., Nakaichi, M., Okuda, H., Minami, K., and Yamazaki, K. Embryotoxic effects of ethylene glycol monomethyl ether in mice. Toxicology 30: 335–343 (1981).
4. Nelson, B. K., Setzer, J. V., Brightwell, W. S., Mathinos, P. R., Kuczuk, M. H., and Weaver, T. E. Comparative inhalation teratogenicity of four industrial glycol ether solvents in rats. Teratology 25: 64A (1982).
5. Tinston, D. J., Doe, J. E., Godley, M. J., Head, L. K., Killick, M. Litchfield, M. H., and Wickramaratne, G. A. Ethylene glycol monoethyl ether (EE): teratogenicity study in rats. Imperial Chemical Industries PLC, Report No. CTL/P/761 to Chemical Manufacturers Association, 1983.
6. Tinston, D. J., Doe, J. E., Thomas, M., and Wickramaratne, G. A. Ethylene glycol monoethyl ether: inhalation teratogenicity study in rabbits. Imperial Chemical Industries PLC, Report No. CTL/P/776 to Chemical Manufacturers Association, 1983.
7. Tinston, D. J., Doe, J. E., Killick, M. E., and Thomas, M. R. Ethylene glycol monoethyl ether acetate (EEAc): inhalation teratogenicity study in rabbits. Imperial Chemical Industries PLC, Report No CTL/P/840 to Chemical Manufacturers Association, 1983.
8. Doe, J. E., and Tinston, D. J. Novel chambers for long term inhalation studies. In: Proceedings of the Inhalation Toxicology and Technology Symposium, Kalamazoo, MI, October 23–24 1980 (B. K. J. Leong, Ed.), Ann Arbor Science Publishers Inc., 1981.
9. Wilson, J. G. Methods for administering agents and detecting malformations in experimental animals. In: Teratology: Principles and Techniques (J. G. Wilson and J. Warkany, Eds.), University of Chicago Press, Chicago, 1965.
10. Staples, R. E., and Schnell, V. L. Refinements in rapid clearing technique in the KOH-Alizarin Red S method for fetal bone. Stain Technol. 39: 61–63 (1964).
11. Stenger, E. G., Aeppli, L., Muller, D., Deheim, E., and Thomann, P. The toxicology of ethylene glycol mono ethyl ether. Arzneim.-Forsch. 21: 880–883 (1971).
12. Kimmel, C. A., and Wilson, J. G. Skeletal deviations in rats: malformations or variations? Teratology 8: 309–316 (1973).